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14. ABSTRACT
This report reviews the third year of research on the diagnostic utility of psychophysiological indicthat may predict the current and future functional efficiency of the soldier. The research focuses especially on the measurement of cerebral bloodflow velocity (CBFV) using transcranial Doppler sonography (TCD), together with additional indices including salivary cortisol and subjective state. Two studies at the University of Cincinnati demonstrated that CBFVdeclines during cognitive vigilancand during simulated driving, extending prior results from sensory vigilance tasks. In addition, phasBloodflow responses to a short task battery predicted cognitive vigilance. Predictive validity was increased by including subjective state measures in a multivariate model. Research at Georgia State University, employing simulated military tasks representing sentry duty, peacekeeping operations,andtactical decision making. These studies confirmed that CBFV correlates with various performance indices, indicating that the technique may have diagnostic utility not just for vigilance, but also fmilitary decision-making. Attentional skills and eye movement indices were also found to have diagnostic utility. The report concludes with a summary of the main findings from the three years of research, and recommendations for future studies to translate the research into applied techniques fodiagnostic monitoring and prediction in military environments.

15. SUBJECT TERMS
Mesenchymal stem cells, prostate cancer, targeted gene delivery, gene therapy

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Introduction

Prostate cancer metastases, especially bone metastases, are the major reason account for high mortality of advanced prostate cancer as they can not be reached by any currently used regimens without detrimental side effects to the patients. Even though the exact mechanism of preferential prostate cancer bone metastasis has not yet been well understood, it is speculated that the migration and establishment of these cancer cells in the bone compartment is contributed by the stimulatory and supportive roles of bone marrow stroma cells or mesenchymal stem cells (MSC). We thus hypothesize that targeting the tumor supportive stroma cells via MSC would represent one promising avenue for our long-term goal of developing an innovative non-invasive approach for treating metastatic prostate cancers.

Body

This research project has not been modified from the previously approved proposal and thus the results are presented in accordance with the proposed tasks. Overall, we had accomplished majority of the planned experiments for tasks 1 and 2 as outlined in the following.

Task 1. To examine the migration and distribution of GFP gene marked human mesenchymal stem cells within subcutaneous and metastatic LuCap 23.1 tumor in SCID mice and their supportive role in forming tumor-stroma mass and neovasculature.

a. Determine the distribution of GFP transduced human mesenchymal stem cells (MSC) in coinjected subcutaneous LuCaP23.1 tumor nodule and characterization of GFP^+ cell population.

We have re-established engineered MSC and human prostate cancer LNCap cell lines and used them for in vitro and in vivo studies. We observed that subcutaneous inoculation of LNCap alone to immune incompetent SCID or nude mice could not establish tumor growth regardless of number of tumors injected. However, when LNCap cells were co-inoculated with human MSC, tumor growth was successfully established. More importantly, immunofluorescent staining against the human factor VIII expressing cells (red, a specific endothelial cell marker) and GFP expressing cells (green) in established LNCap-DsRed tumors revealed that MSC population appear to lead the neovasculature development. These results support our hypothesis that MSC serves as a supportive population for tumor establishment and growth (attached IMPaCT poster).

b. Determine the migration and distribution of GFP marked MSC in LuCaP 23.1 bone metastases and characterization of GFP^+ cell populations.
We also inoculated prostate cancer alone or in combination with MSC to tibia bone cavity and examined their establishment in SCID mice. At the early time points, i.e. the first 2 weeks post-tumor inoculation, no obvious tumor mass was identified in the bone sections when either prostate cancer was inoculated alone or in combination with MSC. However, at about 3 - 4 weeks post-inoculation, tumor establishment in the marrow cavity was evident if LNCap was co-inoculated with MSC, but not LNCap alone. This tumor mass usually took over the entire marrow cavity within 5 weeks and started to invade the bone component (attached IMPaCT poster).

c. Examine the migration and involvement in neovasculature of intravenously injected GFP-MSC in pre-established bone metastatic LuCaP 23.1.

Not yet accomplished.

Task 2. To examine the therapeutic efficiency in selective elimination of subcutaneous and bone metastatic LuCaP 23.1 upon pro-drug administration and bystander-effect mediated destruction of tumor-stroma mass with modified MSC carrying suicide HSV-TK gene.

a. Construct lentiviral vector carrying HSV-TK (suicide) gene under the control of a hypoxia inducible promoter OBHRE.

Construction of lentiviral vector containing the HSV-TK gene was accomplished and used in the subsequent experiments as planned. The effectiveness of MSC transduced with HSV-TK in turning prodrug to cytotoxic chemical for killing LNCap cells has been tested in culture as shown in the attached IMPaCT poster.

b. Examine the effects of OBHRE-HSV-TK transduced MSC in GCV mediated killing of subcutaneous LuCaP 23.1 tumors.

The effectiveness of these HSV-TK transduced MSC in eliminating subcutaneously inoculated LNCap cells was examined in the same mouse inoculated with LNCap + MSC-TK on one flank and LNCap +MSC-GFP on the opposite flank. When mice were treated with GCV prodrug, LNCap growth was significantly reduced only on the side where MSC-TK was co-inoculated, but not on the side where MSC-GFP was inoculated (attached IMPaCT poster). In contrast, when mice were treated with PBS, LNCap growth, either on the side co-inoculated with MSC-TK or MSV-GFP, was not altered (IMPaCT poster).

c. Determine specific CaP killing through TK gene modified MSC in LuCap32.1 metastasized to bone compartment.

On going.

Key Research Accomplishments

Growth of human prostate cancer in the presence or absence of human MSC was evaluated in immune incompetent SCID and nude mice;

We concluded from subcutaneously or intra-tibially inoculated human prostate cancer cells that human MSC provide essential support for their establishment and growth.

Lentiviral vector carrying suicide gene HSV-TK was constructed and their efficacy in eliminating co-cultured prostate cancer cells was confirmed.

Effectiveness of MSC-TK in controlling LNCap tumor growth subcutaneously in mice was also confirmed.

Reportable outcome

The experimental results supported by this grant were presented during the 2007 Atlanta IMPaCT meeting.

Conclusions

As outlined above, we have made major progresses towards accomplishing this project as proposed. Most importantly, our preliminary experiments demonstrated that our hypothesis is correct and we are in the process of obtaining more supporting results to write a scientific paper for publication in the near future.

References

N/A.

Appendices

1. Abstract submitted to the 2007 Atlanta IMPaCT meeting
2. Poster presented during the 2007 Atlanta IMPaCT meeting.

Targeted Eradication of Prostate Cancer Mediated by Engineered Mesenchymal Stem Cells

Prostate cancer (CaP) metastases, especially bone metastases, are the major reason account for high mortality of advanced prostate cancer. There is an urgent need in developing new approaches to targeted eliminate metastatic prostate cancer in the bone and other tissues to improve quality of life and survival for patients with advanced disease. It is thought that preferential CaP bone metastasis is due to the stimulatory and supportive roles of bone marrow stroma cells. Thus, targeting tumor-stroma interaction represents promising therapeutic strategy for reducing and eliminating CaP metastases. Bone marrow stroma cells are derived from a special type of cell population called mesenchymal stem cells (MSC). These cells possess multipotent self-renewable potentials and are responsible for replacing and repairing multiple mesenchymal tissues, such as bone, cartilage, adipose and connective tissues. Early studies demonstrated that mouse marrow stroma cell line enhances human CaP cell establishment and metastases in athymic nude mice. We, thus, hypothesize that human MSCs preferentially migrate to CaP metastatic sites and provide supportive environment for tumor establishment and angiogenesis. We further hypothesize that engineering these tumor supportive MSCs to express a cytotoxic gene will allow us to targeted eliminate metastatic CaP within the tumor-MSC/stroma environment.

We tested our hypotheses using human prostate cancer LNCaP cell line and immune deficient SCID mice. LNCaP is a slow growing cell line in culture and could not be established in SCID mice in the absence of additional supportive cell populations. However, when we culture LNCaP together with human MSCs, their growth in culture is markedly accelerated. Furthermore, co-inoculation of human MSCs with LNCaP to SCID mice, either subcutaneously or intra-tibially, warrants the establishment of LNCaP tumor in SCID mice. These results demonstrate that MSCs indeed provide a supportive stroma environment for tumor establishment and growth. We next examined whether engineered MSCs carrying a suicide gene, HSV-TK (called TK-MSC), mediate killing of LNCaP cells upon administration of non-toxic pro-d rug ganciclovir (GCV). When LNCaP cells were co-cultured with TK-MSC in the presence of GCV, both MSCs and LNCaPs were killed in a dose dependent manner within a week. In contrast, the same amount of GCV did not affect the survival and growth of LNCaP cells or GFP expressing MSCs (GFP-MSC) when they were co-cultured. Therefore, the observed LNCaP elimination in TK-MSC culture was the results of conversion of GCV to toxic product by the TK gene within TK-MSCs. This TK-MSC targeted LNCaP killing was also observed in subcutaneously growing LNCaP in SCID mice that were treated with GCV, but not in mice carrying LNCaP with GFP-MSC. We are in the process of evaluating whether this TK-MSC targeted LNCaP killing would be effective for tumor cells growing in the tibia of SCID mice.

IMPACT: This proof of principle study explores whether we can use engineered human MSC as a tumor targeting vehicle to treat prostate cancer metastases. Positive results from this study will facilitate the development of new clinical translatable CaP treatment protocols.

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Targeted Eradication of Prostate Cancer Mediated by Engineered Mesenchymal Stem Cells

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ABSTRACT

Bone metastasis has been a hallmark of advanced prostate cancer (CaP) as about 80% of clinical CaP metastases possess bone component. Mesenchymal stem cells (MSC) are specific type of multipotent self-renewable cells existing in the bone marrow, which are responsible for replacing and repairing multiple mesenchymal tissues and may provide fertile environment for CaP establishment and metastases. Early studies demonstrated that mouse marrow stroma cell line enhances human CaP cell establishment and metastases in athymic nude mice. We, thus, hypothesize that human MSCs preferentially migrate to CaP metastatic sites and provide supportive environment for tumor establishment and angiogenesis. We further hypothesize that engineering the tumor supportive MSCs to express a cytotoxic gene will allow us to targeted eliminate metastatic CaP within the tumor-MSC/stroma environment.

We tested our hypotheses using human prostate cancer LNCaP cell line and immune deficient SCID mice. LNCaP is a slow growing cell line in culture and barely grow in SCID mice in the absence of additional supportive cell populations. However, when we culture LNCaP together with human MSCs, their growth in culture is markedly accelerated. Furthermore, co-inoculation of human MSCs with LNCaP to SCID mice, either subcutaneously or intra-tibially, warrants the establishment of LNCaP tumor in SCID mice. These results demonstrate that MSCs indeed provide a supportive stroma environment for tumor establishment and growth. We next examined whether engineered MSCs carrying a suicidal gene, HSV-TK (called MSC-TK), mediate killing of LNCaP cells upon administration of non-toxic pro-drug ganciclovir (GCV). When LNCaP cells were co-cultured with MSC-TK in the presence of GCV, both MSCs and LNCaPs were killed in a dose dependent manner within a week. In contrast, the same concentration of GCV did not affect the survival and growth of LNCaP cells or GFP expressing MSCs (MSC-GFP) when they were co-cultured. Therefore, the observed LNCaP elimination in MSC-TK culture was due to enzymatic conversion of GCV to cytotoxic product by TK in MSC-TK cells and corresponding bystander killing. This MSC-TK targeted LNCaP killing was also confirmed in SCID mice. Specifically, the growth of LNCap tumor co-inoculated with MSC-TK in SCID mice was significant suppressed when they were treated with GCV. In contrast, the growth of LNCaP tumor co-inoculated with MSC-GFP on the opposite flank of the same mouse was not affected by GCV treatment. We are now evaluating whether this MSC-TK targeted LNCaP killing would be effective for eliminating LNCap cells or slowing down tumor progression in an experimental bone metastasis setting in SCID mice.

IMPACT: This proof of principle study explores whether we can use engineered human MSC as a tumor targeting vehicle to treat prostate cancer metastases. Positive results from this study will facilitate the development of new clinical translatable CaP treatment protocols.

INTRODUCTION

Prostate cancer (CaP) metastases, especially bone metastases, are the major reason account for high mortality of advanced prostate cancer. There is an urgent need in developing new approaches to targeted eliminate metastatic prostate cancer in the bone and other tissues to improve quality of life and survival for patients with advanced disease. It is thought that preferential CaP bone metastasis is due to the stimulatory and supportive roles of bone marrow stroma cells. Thus, targeting tumor-stroma interaction represents promising therapeutic strategy for reducing and eliminating CaP metastases.

So far, the exact mechanism of preferential CaP bone metastasis has not yet been well understood, it is suggested that the successful "seeding" of CaP cells migrated to the bone compartment is, at least partly, due to the stimulatory and supportive roles of bone marrow stroma cells in providing a favorable and fertile environment. This is supported by the fact that growth and survival of CaP in culture is enhanced by the presence of bone marrow stroma cells and growth factors they produced either in culture or *in vivo*. In addition, it has been demonstrated experimentally and clinically that this enhancement is mediated through reciprocal interaction of stroma, tumor epithelial, and endovascular precursors to stimulate new blood vessel formation - angiogenesis. This reciprocal interaction is vital for CaP tumorgenesis, metastases and angiogenesis. Thus, tumor-stroma interaction represents promising therapeutic targets for reducing and eliminating CaP metastases. To utilize the property of stroma cells, which are derived from a special type of cell population called mesenchymal stem cells (MSC), for active elimination of tumor metastases, it is plausible to employ engineered MSC to selectively destruct tumor-stroma interaction via cytotoxic gene mediated killing of stroma cells and concurrent tumor killing via bystander effects.

METHODS

Lentiviral vector transduction of human MSC and LNCap cells

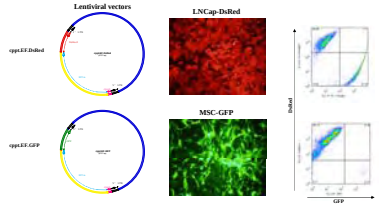

Fig. 1. Lentiviral vectors, cppt.EF.DsRed and cppt.EF.GFP efficiently transduce human prostate cancer LNCap cells (top row) and human mesenchymal stem cells (MSC, bottom row). Transgene expression can be examined via fluorescence microscopy (middle column) or flow cytometry (right column).

Examination of tumor establishment and growth post-subcutaneous inoculation using image analysis system

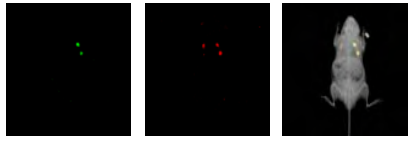

Fig. 2. Detection of LNCap-DsRed and MSC-GFP post-subcutaneous inoculation. Nude mouse was inoculated with 1X 10^6 LNCap-DsRed cell alone (right flank) and 1X10^6 LNCap mixed with 1X 10^6 MSC-GFP cells (left flank). It was anesthetized and placed in a Kodak In-Vivo Imaging System FX. GFP (left panel) and DsRed (middle panel) fluorescent signals were acquired with appropriate filters and represented in pseudo-color. X-ray radiogram was also acquired to visualized the physical location of tumor inoculation and superimposed with GFP and DsRed signals (right panel). Tumor growth can be followed for days and weeks post-inoculation.

Examination of LNCap establishment and growth in the marrow cavity after intra-tibia injection

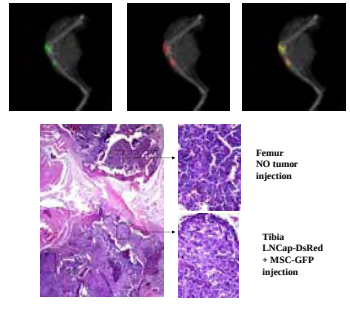

Fig. 3. SCID or Nude mice were inoculated with 1 X 10^5 LNCap-DsRed along with 1X10^5 MSC-GFP intra-tibially. The GFP (top left) and DsRed (top middle) signals were acquired using the Kodak In-Vivo Imaging System FX immediately after injection. Tumor establishment and expansion in the marrow compartment could be followed overtime. Massive tumor growth and damage to bone compartment in tibia was further confirmed via histological examination 4 weeks after tumor inoculation (bottom panels).

RESULTS

Human MSC promote the growth of human prostate cancer LNCap cells in culture

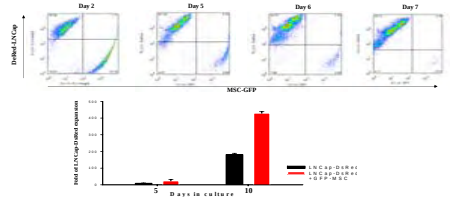

Fig.4 LNCap-DsRed cells (1X 10^6) were seeded in a 6-well plate alone or along with 1X10^4 MSC-GFP. At various time after co-culture, cells were harvested and counted for total cell number. At same time, they were analyzed via flow cytometry for the composition of LNCap-DsRed and MSC-GFP cells (top panels). The corresponding total number of LNCap-DsRed cells at various time after co-culture was then calculated and plotted against number of LNCap-DsRed cells cultured alone.

Human MSCs support the establishment and growth of LNCap cells in immune deficient SCID mice

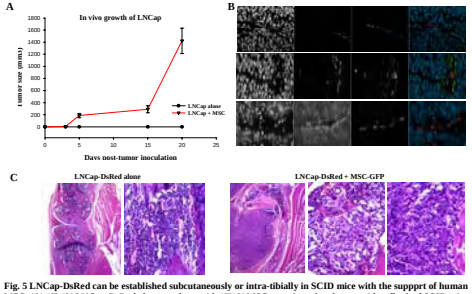

Fig. 5 LNCap-DsRed can be established subcutaneously or intra-tibially in SCID mice with the supppprt of human MSC. (A) 1X 10^6 LNCap-DsRed alone or along with 1X10^6 MSC were inoculated s.c. to either flank of SCID mice. Tumor growth and the size was examined every 5-7 days. (B) The existence of human factor VIII expressing cells (red, a specific endothelial cell marker) and GFP expressing cells (green) in established LNCap-DsRed tumors in SCID mice co-inoculated with MSC-GFP (top and middle panels) or unmodified MSC (bottom panel) was examined via immunofluorescent staining. (C) Histological examination of tumor establishment (right panels) or lack of tumor (left panels) in the marrow compartments 4 weeks after intra-tibia injection of 1X 10^5 LNCap-DsRed alone to the left hind leg (left panels) or 1X 10^5 LNCap-DsRed mixed with 1 X 10^5 MSC-GFP to the right hind leg (right panels) of the same SCID mouse.

Engineered human MSCs expressing HSV-TK gene support LNCap growth which in turn can be utilized for LNCap elimination upon addition of pro-drug GCV

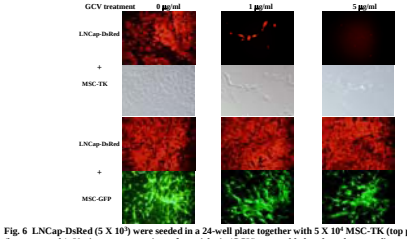

Fig. 6 LNCap-DsRed (5 X 10^3) were seeded in a 24-well plate together with 5 X 10^4 MSC-TK (top panels) or MSC-GFP (bottom panels). Various concentrations of ganciclovir (GCV) were added to the culture medium starting from day 2 after seeding. Culture medium and GCV was replaced every day and cell viability was monitored via fluorescent microscopy. The images were representative of 4 separate experiments at 7-10 days post-GCV treatment.

RESULTS

GCV treatment of mice carrying LNCap-DsRed tumors specifically prevents their progression when supportive MSC expresses HSV-TK

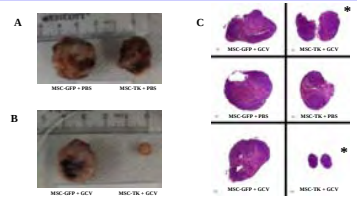

Fig. 7 LNCap-DsRed (1 X 10^6) mixed with MSC-GFP (1 X 10^6) were inoculated to the right flank of SCID mice, whereas LNCap-DsRed (1 X 10^6) mixed with MSC-TK (1 X 10^6) were inoculated to the left flank of the same mouse. When tumor reached palpable size, usually 5-10 days post-inoculation, tumor bearing mice were treated with i.p. injections of either PBS (A) or GCV (B, 30 mg/Kg body weight) every 12 hours for 14 days. The mice were euthanized at the end of GCV treatment. Tumor size, morphology and histology (C) were examined. * due to the small tumor size in MSC-TK group treated with GCV, each tumor was halved and placed side-by-side before sectioning to obtain more coverage of tumor area for better representation.

GCV treatment significantly suppressed LNCap growth when MSC-TK was used as supportive population but not LNCap supported by MSC-GFP cells

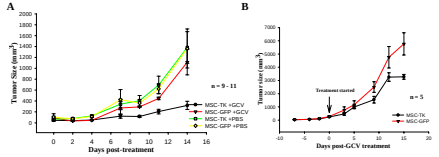

Fig. 8 (A) LNCap-DsRed (1 X 10^6) mixed with MSC-GFP (1 X 10^6) were inoculated to the right flank of SCID mice, whereas LNCap-DsRed (1 X 10^6) mixed with MSC-TK (1 X 10^6) were inoculated the left flank of the same mouse. When tumor reached palpable size (<100 mm^3), usually 5-10 days post-inoculation, tumor bearing mice were treated with i.p. injections of either PBS (green and yellow lines) or GCV (black and red lines, 30 mg/Kg body weight) every 12 hours for 14 days. The size of tumor was measured twice a week and the tumor volume was calculated as $axb^2/2$. (B) When GCV treatment was delayed till tumor reached size of larger than 250 mm^3 or only a low percentage of MSC were carrying HSV-TK gene, suppression of tumor progression was compromised although still significantly better than LNCap co-injected with MSC-GFP.

CONCLUSIONS

This study has demonstrated:

1. Human MSC can facilitate growth and establishment of human LNCap cells in culture and in immune deficient SCID mice;
2. The supportive feature of human MSC can be utilized to our advantages for suppressing tumor progression or resulting in tumor elimination;
3. Therapeutic effectiveness of this approach can be further enhanced by increases in input MSC cells, especially with high percentage of MSC cells expressing HSV-TK gene.

ACKNOWLEDGEMENTS

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